CLEAN VERSION OF AMENDED CLAIMS - 50915

Q1 4. A nucleic acid sequence coding for a monooxygenase according to claim 1.

11. A process as claimed in claim 9, where the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from Bacillus megaterium having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43. 48-52. 67-70, 330-335, 352-356, 73-82 and 86-88.

Q2

13. A process for microbiological oxidation of optionally substituted mono- or polynuclear aromatics, straight-chain or branched alkanes or alkenes, or optionally substituted cycloalkanes or cycloalkenes, which comprises

a1) culturing the recombinant cytochrome P450-producing microorganism as claimed in claim 7 in a culture medium, in the presence of an exogenous or intermediately formed substrate; or

Q3

a2) incubating a substrate-containing reaction medium with a cytochrome P450 monooxygenase derived from cytochrome P450 monooxygenase BM-3 from Bacillus megaterium having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43. 48-52. 67-70, 330-335, 352-356, 73-82 and 86-88; and

b) isolating the oxidation product formed or a secondary product thereof from the medium;

where the monooxygenase mutant Phe87Val is not excluded.

CLEAN VERSION OF AMENDED CLAIMS - 50915

15. (cancel)

16. A process as claimed in claim 13, where the cytochrome P450 monooxygenase has at least one of the following mono- or polyamino acid substitutions:

a) Phe87Val;

b) Phe87Val, Leu188Gln; or

c) Phe87Val, Leu188Gln, Ala74Gly.

17. A process as claimed in claim 9, wherein, as exogenous substrate, at least one compound selected from the groups a) to d) of compounds defined above is added to a medium and the oxidation is carried out by enzymatic reaction of the substrate-containing medium in the presence of oxygen at a temperature of approximately 20 to 40°C and a pH of approximately 6 to 9, where the substrate-containing medium additionally contains an approximately 10- to 100-fold molar excess of reduction equivalents based on the substrate.

19. A process for the microbiological production of indigo and/or indixubin, which comprises

a1) culturing a recombinant microorganism which produces an indole-oxidizing cytochrome P450 in a culture medium, in the presence of exogenous or intermediately formed indole; or

a2) incubating an indole-containing reaction medium with an indole-oxidizing cytochrome P450 monooxygenase; and

b) isolating the oxidation product formed or a secondary product thereof

from the medium.

22. A process as claimed in claim 20 , where the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from Bacillus megaterium having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43. 48-52. 67-70, 330-335, 352-356, 73-82 and 86-88, including the substitution Phe87Val.

23. A process as claimed in claim 22, where the monooxygenase has at least one of the following mono- or polyamino acid substitutions:

a) Phe87Val;

b) Phe87Val, Leu188Gln; or

c) Phe87Val, Leu188Gln, Ala74Gly.

24. A bioreactor comprising the cytochrom P450 monooxygenase as claimed in claim 1 or a recombinant microorganism transformed by a vector comprising an expression construct comprising a nucleic acid sequence coding for the cytochrom P450 monooxygenase of claim 1 in immobilized form.

25. (cancel)

26. (cancel)

MARKED VERSION OF AMENDED CLAIMS - 50915

4. (amended) A nucleic acid sequence coding for a monooxygenase according to [one of the preceding claims] claim 1.

11. (amended) A process as claimed in claim 9 [or 10], where the monooxygenase is [a mutant as claimed in any of claims 1 to 3, including the mutant Phe87Val] derived from cytochrome P450 monooxygenase BM-3 from Bacillus megaterium having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43. 48-52. 67-70, 330-335, 352-356, 73-82 and 86-88.

13. (amended) A process for microbiological oxidation of [a compound as defined in claim 1 b), c) or d)] optionally substituted mono- or polynuclear aromatics, straight-chain or branched alkanes or alkenes, or optionally substituted cycloalkanes or cycloalkenes, which comprises

a1) culturing [a] the recombinant cytochrome P450-producing microorganism as claimed in claim 7 [or 8] in a culture medium, in the presence of an exogenous or intermediately formed substrate; or

a2) incubating a substrate-containing reaction medium with a cytochrome P450 monooxygenase [as claimed in any of claims 1 to 3] derived from cytochrome P450 monooxygenase BM-3 from Bacillus megaterium having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43. 48-52. 67-70, 330-335, 352-356, 73-82 and 86-

MARKED: ***VERSION OF AMENDED CLAIMS - 50915***

15. (cancel)

16. (amended) A process as claimed in claim [15] 13, where the [mutant] cytochrome P450 monooxygenase has at least one of the following mono- or polyamino acid substitutions:

a) Phe87Val;

b) Phe87Val, Leu188Gln; or

c) Phe87Val, Leu188Gln, Ala74Gly.

17. (amended) A process as claimed in [any of claims] claim 9 [to 16], wherein, as exogenous substrate, at least one compound selected from the groups a) to d) of compounds defined above is added to a medium and the oxidation is carried out by enzymatic reaction of the substrate-containing medium in the presence of oxygen at a temperature of approximately 20 to 40°C and a pH of approximately 6 to 9, where the substrate-containing medium additionally contains an approximately 10- to 100-fold molar excess of reduction equivalents based on the substrate.

19. (amended) A process for the microbiological production of indigo and/or indixubin, which comprises

a1) culturing a recombinant microorganism which produces an indole-oxidizing cytochrome P450 in a culture medium, in the presence of exogenous or intermediately formed indole; or

a2) incubating an indole-containing reaction medium with an indole-oxidizing

MARKED VERSION OF AMENDED CLAIMS - 50915

a1) culturing a recombinant microorganism which produces an indole-oxidizing cytochrome P450 in a culture medium, in the presence of exogenous or intermediately formed indole; or

a2) incubating an indole-containing reaction medium with an indole-oxidizing cytochrome P450 monooxygenase; and

b) isolating the oxidation product formed or a secondary product thereof from the medium[;].

22. (amended) A process as claimed in claim 20 [or 21], where the monooxygenase is [a mutant as claimed in any of claims 1 to 3] derived from cytochrome P450 monooxygenase BM-3 from Bacillus megaterium having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88, including the [mutant] substitution Phe87Val.

23. (amended) A process as claimed in claim 22, where the [mutant] monooxygenase has at least one of the following mono- or polyamino acid substitutions:

a) Phe87Val;

b) Phe87Val, Leu188Gln; or

c) Phe87Val, Leu188Gln, Ala74Gly.

24. (amended) A bioreactor comprising [an enzyme] the cytochrom P450 monooxygenase as claimed in [one of claims] claim 1 [to 3] or a recombinant

MARKED VERSION OF AMENDED CLAIMS - 50915

microorganism [as claimed in one of claims 7 or 8] transformed by a vector comprising an expression construct comprising a nucleic acid sequence coding for the cytochrom P450 monooxygenase of claim 1 in immobilized form.

25. (cancel)

26. (cancel)